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EXTRACTION AND ANALYTICAL STUDY OF SALVINORIN A FROM LEAVES OF *SALVIA DIVINORUM*

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Brain-imaging studies in animals provided evidence that salvinorin A biostructure, in addition to hallucinations and impairment of motor function in humans, could become a popular drug. *Salvia divinorum* samples were analyzed by gas chromatography, after being dried at 40°C. For extraction was used analytical grade purity acetone and recrystallisation was achieved with methanol of chromatographic purity. Preliminary processing of crude extract allowed enriching the final solution composition in salvinorin A. The large number of signals showed that under ionization an advanced fragmentation of the molecule occurred. By the advanced fragmentation of *S. divinorum* it was obtained a sample image, with the mass spectrometer, which may constitute a specific footprint of the component salvinorin A.

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1. Introduction

Salvia species are reported in literature for possessing biological activities such as antibacterial, antifungal and antioxidant properties [1, 2]. Several species of *Salvia* are known at this moment, but only *Salvia divinorum* contains psychoactive compounds and this is why this species is the subject of many studies and debates [3]. The effects are hard to obtain by smoking the dried leaves, but leaf extract can cause dramatic effects, sometimes fear or state of captivity [4]. The active substance in *S. divinorum*, salvinorin A, is a chemical compound whose molecule contains only carbon, hydrogen and oxygen, not being an alkaloid (meaning that it does not contain a nitrogen base).

Systematic name is (2S,4aR,6aR,7R,9S,10aS,10bR) -9 -(acetyloxy) -2 -(3-furanyl) dodecahydro -6a,10b-dimethyl-4,10-dioxo-2H-naphtho [2,1-c] pyran- 7-carboxylic acid methyl ester and CAS number: 83729-01-5. Salvinorin A is a hallucinogen diterpene. The chemical composition of vegetal material and consequently the effects obtained depend on plant part used, the stage of plant development and/or type of biostructures [5]. Systematic studies indicated that the salvinorin concentration of the mature plants ranges between 0.89-3.7 mg/g [6] dried leaves (corresponding to an average of 0.245%). According to the National Institute on Drug Abuse (NIDA), salvinorin A is a potent and selective inhibitor to nerve cells called receptors κ-opioid (kappa-opioid), agonist receptor [7].

Opioids bind to specific receptors whose activity is responsible for the effects of these physiological peptides. The affinity of various opioid diterpenes for one or other type of receptors and their capability is diverse. Opioid analgesics act agonist at the level of receptor-specific

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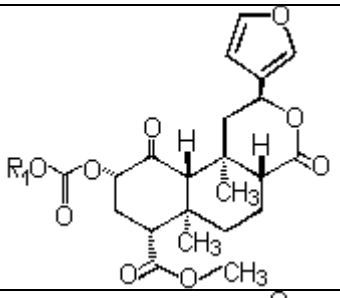
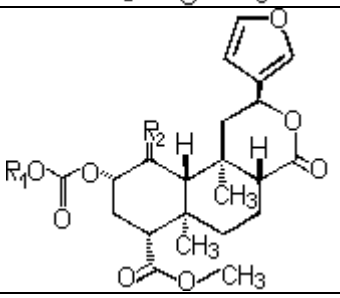
opioids which correspond to endogenous opioid peptides, and diterpenes. Specific opioid receptors are of two interconvertible forms, one with high affinity for agonists, other for antagonists [8, 9].

Ions of Na^+, at a concentration of 1 mmol/L *in vitro*, enable the second state. Some researchers pursuing all effects produced by some opioids and phenomena influencing the development of cross-tolerance, addiction and withdrawal syndrome, described three types of specific opioid receptors μ–"miu" ("M" from morphine), k "kappa" ("k" from ketociclazocin) and σ-"sigma" ("S" from SKF10047). After discovery of enkefalines, researchers revealed other receptors, in mice, such as: type E δ ("delta" - "D" from deferens) and later other types: specific opioid receptors ε ("epsilon" - "E" from endorphin), RO ι ("iota" - "I" from the rabbit intestine), RO λ ("lambda" - "L" from laudanum).

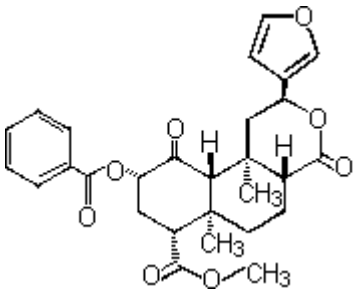
More recently, were discovered specific opioid receptors ζ ("zeta" - "Z" from zoe / life) highlighting that opioids have inhibitory effect on neuronal cell tumors [10, 11]. It is not known whether *S. divinorum* produces mental or physical addiction. The effect is a state of drunkenness much different than alcohol [12]. The issue of effects after consumption of *S. divinorum* based products and the addiction threat is still controversial in the literature [13]. The dried leaves of the plant or leaf extracts are utilised for different biostructures intended for recreational use [14].

The main components of *S. divinorum* leaf, described in the literature, are salvinorin A, salvinorin B, salvinorin C, salvinorin D, salvinorin E, salvinorin F, salvinorin G and salvinorin H (Table 1).

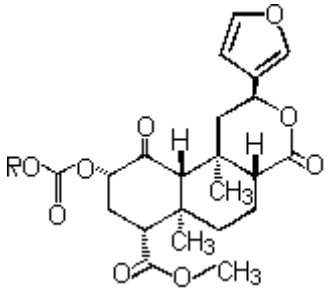
Table 1. Natural compounds isolated from S. divinorum

Compound	**Chemical structure**	**R1**	**R2**	**Activity**
Salvinorin A		$–O–CO–CH_3$	–	active
Salvinorin B		–OH	–	inactive
Salvinorin C		$–O–CO–CH_3$	$–O–CO–CH_3$	unknown
Salvinorin D		–OH	$–O–CO–CH_3$	inactive
Salvinorin E		$–O–CO–CH_3$	–OH	inactive
Salvinorin F		–H	–OH	unknown
Salvinorin G		=O	$–OCOCH_3$	unknown
Salvinorin H		–OH	–OH	unknown

The most extensively studied among these compounds are salvinorin A and salvinorin B. In order to gain selectivity of action on opioid receptors κ [15], μ and δ [16], semi-synthetic derivatives were designed (Figure 1) such as 2-ethoxymethyl salvinorin B, 2-methoxyethyl salvinorin B and herkinorin [17]. 2-ethoxymethyl salvinorin B proved to be the most selective opioid κ agonist receptor among the salvinorin representatives (agonist approximately 3000-fold more selective of opioid κ receptor [18] than of μ and δ receptor).



Herkinorin



R= CH_3 salvinorin B methoxymethyl ether
R= C_2H_5 salvinorin B ethoxymethyl ether

Fig. 1. Herkinorin and derivatives of salvinorin B

Since the plant components, especially salvinorin A, are used as addictive biostructures [19], it is required the identification and quantitative determination of the compound salvinorin A in various ethnobotanical products.

The aim of this study was to verify the possibility of isolating the biostructure of interest by a process of extraction and recrystallization, by chromatographic separation and identification by mass spectrometry.

2. Materials and methods

S. divinorum leaves were obtained and used only for research purposes. Plant material was harvested from Arges county, Romania. The samples were dried at 40°C. For extraction was used analytical grade acetone and recrystallization was executed from methanol of chromatographic purity. Gaschromatograms were recorded on a HP 5890 Series II Plus instrument, Capillary column (HP-5, film 15 mx 0.25 mm inside diameter), coated on the inside with a stationary phase of 5% phenyl-methyl-siloxane (thickness 0.25 μm). Helium was used as carrier gas.

For separation of compounds, on the chromatographic column was injected a volume of 1 μl methanol solution in splitless mode ("splitless injection") [20]. The injector temperature was maintained at 280°C. Separation was achieved by programmed temperature regime (2 minutes at 25°C initially, and then progressive heat increasing with 30 °C/min to reach the final temperature of 300°C and maintaining this temperature for the next 5 minutes). The effluent from the chromatographic column was introduced into the mass spectrometer (HCT ultraPTM Discovery System, serial, 1047, from Bruker). Monitoring chromatographic separation was performed on a "total ions" regime. MS data records were processed on specialized software tool, "esquire Control, version 10116120" [21, 22].

3. Results and discussion

The primary processing of the sample was carried out according to the scheme shown in Figure 2. The working amount of 224 grams of dried leaves was crushed to a size of about 1 mm and subjected to extraction three times with acetone. Consecutive extracts were combined. Solid residue was removed by filtration and the filtrate was evaporated to dryness. After dissolution in a mixture of ethyl-heptane acetate (volume ratio 50:40), the solution was passed through a layer of activated carbon to remove coloured components. The solution was evaporated to dryness, and the residue was recrystallized three times from methanol. After the final recrystallization the residue was dissolved in methanol. Figure 3 shows the gaschromatogram of the acetone extract purified according to the scheme in Figure 2.

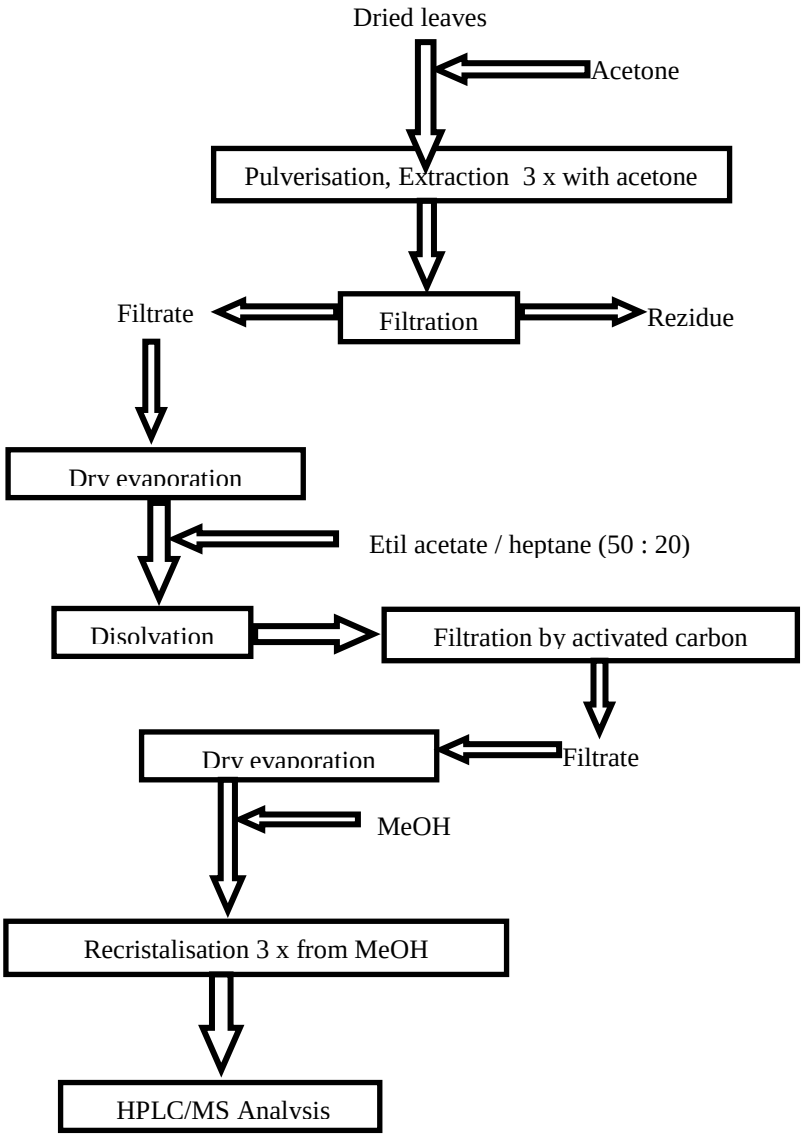


Fig. 2. Stages of the preliminary purification and extraction

It was observed that preliminary processing of crude extract allowed enriching the final solution with salvinorin A. The chromatogram shows sporadic presence of salvinorin B and salvinorin C with very small amounts of other impurities whose identity has not been established yet [23, 24].

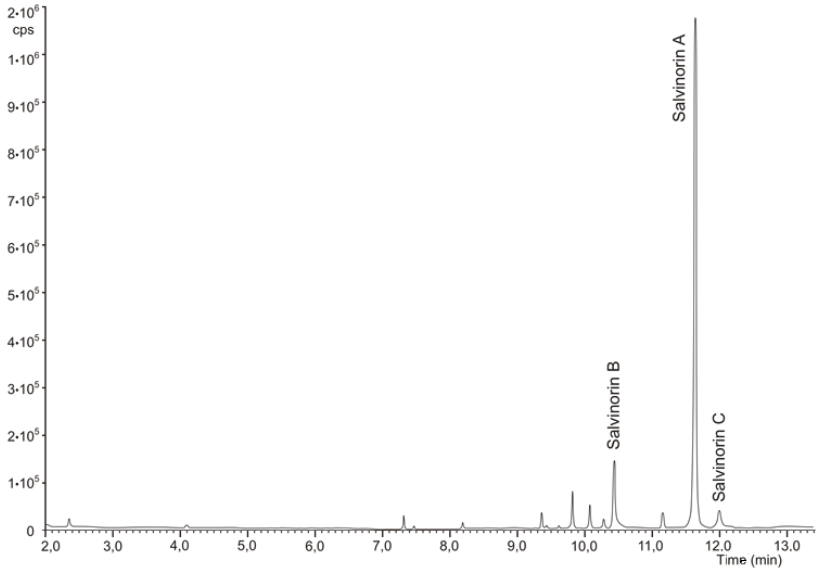


Fig. 3. Gas chromatogram of the purified acetone extract

To confirm the identity of the component that appears in the chromatogram at 11.64 minutes retention time, was recorded the mass spectrum of the fraction in question (Figure 4).

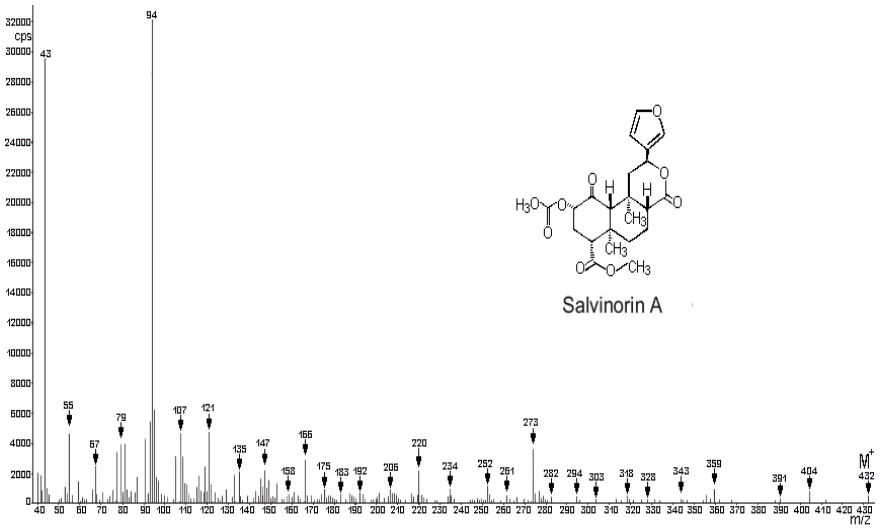


Fig. 4. MS Spectrum of the component at a retention time 11,64 minutes

Mass spectrum obtained by electron ionization of salvinorin A shows substantial fragments at *m/z* 94, 55, 121, 107, 273, 166, 220, 252, 234, 359, 318, 404 and 432. The UV spectrum of the methanol solution of salvinorin A has a peak at 211 nm [25]. Characteristic absorption bands in the infrared spectrum in KBr of salvinorin A are at 3220, 1745, 1735, 1240, and 875 cm^{-1}.

However, for unambiguous identification of powdered vegetal biostructures [26] are recommended further DNA fingerprinting methods [27]. Salvinorin A and other plant diterpenoids are not detected by conventional methods for the evaluation of the drugs [28].

4. Conclusion

Given the ionization conditions it was expected an advanced fragmentation of the molecule, as confirmed by the large number of signals. Due to the advanced fragmentation of the sample the MS image obtained may be a specific footprint of salvinorin A. The mass spectrum is similar to that presented in the literature obtained for pure salvinorin A (reference salvinorin). Based on the results obtained following the procedure described (preliminary preparation, gas chromatographic separation and analysis by mass spectrometry of the component with retention time 11.64 minutes) it can be detected with advanced accuracy the presence of salvinorin A in various ethnobotanical products. *S. divinorum* is not considered a species under control. For a substance to be included in the list of narcotic or psychotropic controlled substances it should present a chemical structure similar to the one of controlled substances. *S. divinorum* is quite different chemically from other controlled substances.

European Monitoring Centre for Drugs and Drug Addiction indicates that in recent years, since were discovered the effects of salvinorin, comparable to those of LSD (in terms of potency, being substances of different classes), this species of sage was put on the list of plants and controlled substances, in several countries in Europe.

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